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PHYSIOLOGICAL OBSERVATIONS UPON A LARVAL EUSTRONGYLIDES

II. The Aerobic Respiration [1]

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In the first paper of this series (von Brand, 1938) the chemical composition of a larval *Eustrongylides* was studied to some extent. It was characterized by a high glycogen and haemoglobin content, whereas only a little fat was found. The different rates of glycogen consumption under aerobic and anaerobic conditions seemed to indicate that the worm, in contrast to *Ascaris* and several other parasitic worms, was adapted to an oxidative rather than an anoxidative type of metabolism. In order to carry the analysis of its metabolic processes one step farther, a study of its respiratory exchange was undertaken.

Material and Methods

All the larvae (probably the larva of *Eustrongylides ignotus* according to a personal communication by Dr. B. G. Chitwood) were extracted from cysts along the mesenteries of *Fundulus heteroclitus* and only medium-sized to large worms (40 to about 120 mgm.) were used. They were washed with saline, dried quickly on filter paper, weighed with an accuracy of 1 mgm. and placed in the respiratory vessels. From one to four worms (100 to 300 mgm. fresh tissue) were used in making a single determination. All the data given below for the gaseous exchange are based on the weight of the living worms immediately after isolation. The salt solutions used varied in the different series, and are mentioned below.

The experiments were carried out with manometers of the Warburg type, using vessels of about 18 cc. capacity, and conducted at a temperature of 37° C. This high temperature was chosen rather than the lower

[1] A contribution from the Department of Biology, The Catholic University of America, Washington, D. C.

[2] The author is indebted to the Elizabeth Thompson Science Fund for a grant towards the purchase of the respiration apparatus used in this investigation.

temperature to which the worms were accustomed in the fish, since the definitive host is quite certainly a warm-blooded animal and the parasite would normally live at the temperature prevailing in the glands of the fore-stomach of aquatic birds after leaving the cyst. To determine the respiratory quotients of the experiments summarized in Tables III and IV, the worms were kept first for two hours in the vessels without KOH, then for the same length of time in the same vessels after addition of KOH. This procedure was permissible, since preliminary experiments had shown that the manometer readings, both for CO_2 and O_2, remained sufficiently constant during such periods. The respiratory quotients of the experiments summarized in Table V, on the other hand, were determined according to Warburg's indirect method, since it was necessary to determine here the RQ's in shorter intervals throughout the course of the experiments. In all cases the readings were taken in half-hour intervals, and the first reading was made 20 minutes after the vessels were inserted in the water-bath.

The readings were taken to the nearest 0.5 mm. The average change in the manometer level was about 8 mm. between two readings in the oxygen consumption experiments. The average error of the single determination from this source alone may, therefore, have been as high as $\pm$ 6 per cent, or even higher, if a similar error for the thermobarometer was added. In the determination of the R.Q., obviously an accumulation of errors occurs; for example, the use of different batches of animals for the determination of the oxygen consumption and carbon dioxide production in those series in which Warburg's indirect method was employed might result in reading errors on three manometers (two for the animals and one for the thermobarometer) besides the biological variation. The exact limits of error of the R. Q. determinations are difficult to evaluate. The "extremes" given in the tables doubtless do not represent the true range of biological variability, but are a summation of this and the errors. The conclusions drawn in this paper are, therefore, based on mean values of a number of experiments for each series, as indicated in the tables. The similarities of the mean values of series conducted under comparable conditions are regarded as sufficient proof that the errors inherent in the single determinations have largely been eliminated and that large differences in the mean values of experiments conducted under different conditions are real.

Results

It was noticed in experiments conducted in another connection that the worms showed in vitro a surprising resistance against changes in the osmotic pressure of their surroundings. It seemed therefore of interest

to study the oxygen consumption in various NaCl concentrations (Table I). The solutions used varied from distilled water to 4 per cent NaCl and with each solution seven individual experiments were performed, each lasting four hours. The initial mean values were fairly similar in all series. They were always somewhat higher than those found in the later stages of the experiments, an observation that will be discussed below. It is apparent that beginning with the third or fourth half-hour period a steady stage was reached in the lower concentrations (0–1.5 per cent NaCl), whereas in the higher concentrations (2.0–4.0 per cent NaCl) a more or less continuous decline occurred. The values of the different series belonging to these two groups have been averaged and the resulting curves are shown in Fig. 1. The steady decline of the O_2 consumption in the higher salt concentrations is obviously an indica-

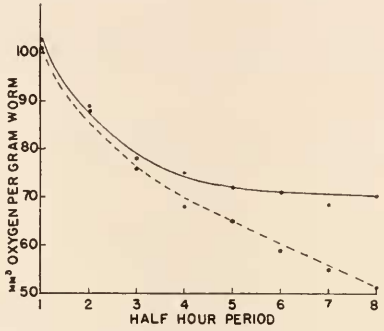

FIG. 1. Oxygen consumption in salines of low and high concentration, mean values. Solid line: 0 to 1.5 per cent NaCl. Broken line: 2 per cent to 4 per cent NaCl.

tion that they are definitely toxic. The fact, however, that variations between 0 and 1.5 per cent NaCl seem not to interfere with the normal respiratory activities, is of interest. This opens up the possibility of studying in future experiments the influence of various substances upon the oxygen consumption irrespective of their osmotic properties.

The fact that these parasites are able to maintain a constant level of metabolism in media of different osmotic pressure has probably a biological basis. Although the life cycle of this species is not known in detail, it can be assumed that the worms live at various stages in surroundings differing, perhaps widely, in molecular concentration.

It should be noted that the variations in the rate of oxygen consumption between different experiments, as indicated by the "extremes" of Table I, were fairly large. Even in the highest salt concentrations, however, some worms were able to maintain a high and fairly regular rate of oxygen consumption. The surprising tolerance of single speci-

mens to a great variety of different media, as judged by their survival in vitro, was frequently noticed and will be discussed in a future paper.

The average oxygen consumption of normal worms was about 140 cu. mm. per gram per hour. It was therefore about twice as great as that reported for *Ascaris* (von Brand, 1934; Harwood and Brown, 1934; Krüger, 1936), but considerably lower than those found in *Setaria equina* (Toryu, 1934) or the hookworm (Harwood and Brown, 1934).

TABLE I

Oxygen consumption of freshly isolated worms kept in salines of different concentrations. Oxygen in cu. mm. per gram of worm. Each mean value is derived from 7 determinations.

Half-hour periods	1	2	3	4	5	6	7	8
0% NaCl								
Mean	94	95	75	83	70	68	71	70
Extremes	51–127	73–112	56–94	56–103	56–88	37–84	55–79	55–83
0.5% NaCl								
Mean	99	80	72	71	73	63	72	78
Extremes	59–130	56–117	40–104	32–123	32–117	21–136	40–143	32–149
1.0% NaCl								
Mean	117	92	81	78	77	80	66	70
Extremes	99–165	46–172	46–178	41–148	48–118	46–137	41–90	40–92
1.5% NaCl								
Mean	103	87	84	67	69	71	64	63
Extremes	63–148	63–134	45–118	28–90	36–92	36–104	27–85	27–89
2.0% NaCl								
Mean	92	82	82	60	60	60	51	55
Extremes	53–161	63–114	39–104	47–82	49–71	49–67	29–65	49–62
3.0% NaCl								
Mean	105	87	86	82	61	63	59	51
Extremes	74–170	60–119	60–112	34–134	17–112	17–100	17–100	17–99
4.0% NaCl								
Mean	105	97	91	61	73	54	56	46
Extremes	83–133	77–109	39–117	19–107	19–115	7–80	14–91	7–83

Unfortunately, a comparison with Fenwick's (1938) data for *Ascaris* larvae is impossible, since he bases his figures on numbers of larvae instead of their weight. Stannard, McCoy, and Latchford (1938) found in *Trichinella* larvae an oxygen consumption of 1.70 cu. mm. per hr. per milligram of dry weight. A calculation of the above *Eustrongylides* figures on this basis, using the previously found figure of 25 per cent dry weight (von Brand, 1938), yields a value of 0.56 cu. mm. Krüger (1940) showed that the rate of oxygen consumption of *Ascaris lumbri-*

coides of various sizes depends rather on surface than on weight. In Table II an analysis of the oxygen consumption of *Trichinella* larvae, *Eustrongylides* larvae and adult females of *Ascaris lumbricoides* has been attempted on this basis. It should be noted that no accurate figures for the fresh weight of *Trichinella* larvae are available in the literature, only data on the dry weight. The fresh weight has been calculated on the basis of 5 per cent dry weight. The conclusion drawn below remains, however, unchanged, even if the weight of the *Trichinella* larvae is calculated on the assumption of 15 per cent dry weight, a figure which is not very likely for these delicate worms. It appears that, contrary to Krüger's findings on individuals of one species, the oxygen consumption of nematodes of very different size is not constant if calculated on the basis

TABLE II

Calculation of oxygen consumption of various helminths on the basis of weight and relative surface.

Species	Weight of 1 worm in mgm.	Relative weight	Surface (Weight $^2/_3$)	Relative surface	O_2 in cu. mm. per worm per hour	Relative O_2 consumption
Trichinella larva (3)	0.007	1	0.037	1	0.0006	1
Eustrongylides larva (4)	82	11700	18.9	511	12.6	21000
Adult Ascaris lumbricoides, female (5)	4780	683000	284	7700	287	478000

[3] The data of Stannard, McCoy and Latchford (1938) for oxygen consumption in saline have been used. The weight of one worm has been calculated assuming 5 per cent dry weight.

[4] The data of the experiments conducted in 1 per cent saline (Table I) have been used.

[5] The data of von Brand (1934) have been used.

of surface. The difference in the rates of oxygen consumption is considerably smaller, although by no means eliminated if the calculation is based on weight. It is recognized that the data are not complete enough to draw general conclusions, but the nematodes appear to be organisms exceptionally well suited for further work along these lines. Many species of intermediate size are available.

The next series was undertaken in order to ascertain the influence of starvation upon the gaseous metabolism. The worms were kept for 7 days in 1 per cent saline in an incubator at 37° C. and daily determinations of the oxygen uptake and carbon dioxide production were performed. In some instances substitute batches, that had starved a similar period under analogous conditions but for which no initial values were

TABLE III

Oxygen consumption and carbon dioxide production of worms starving in 1 per cent saline at 37° C.

Days starving	0	1	2	3	4	5	6	7
Number of determinations	9	7	8	11	11	13	5	12
O_2 cu. mm./gram/hour								
Mean	152	100	113	104	74	82	85	71
Extremes	121–202	63–120	56–142	75–170	57–99	53–138	62–98	48–95
CO_2 cu. mm./gram/hour								
Mean	158	101	122	113	74	82	85	75
Extremes	115–198	57–119	63–171	86–172	56–93	56–138	69–106	48–118
RQ								
Mean	1.07	1.02	1.06	1.10	1.01	1.01	1.01	1.04
Extremes	0.89–1.28	0.89–1.09	0.82–1.18	0.98–1.28	0.77–1.19	0.87–1.11	0.90–1.12	0.83–1.25

available, had to be used during the later days of the starvation period due to the death of worms in some of the original lots. A slow decline in the rate of oxygen consumption and carbon dioxide production occurred under the conditions of this experiment. The respiratory quotient was found to be rather variable, if individual experiments are considered. The mean values for the single days were, however, rather similar and lay always slightly above 1.0. The mean value for the whole series was 1.04 ± 0.013, and similar mean values were also found in the series mentioned below. They are considerably lower than those reported for *Ascaris* (von Brand, 1934, Krüger, 1937) and also somewhat lower than those found in *Trichinella* larvae (Stannard, McCoy and Latchford, 1938). This indicates doubtless that the metabolism of the worm in question is mainly oxidative, but it seems likely that a small amount of anoxidative processes is superimposed on the oxidations.

In an effort to ascertain whether these surmised anoxidative processes are characterized by the production of acids, a similar series of experiments was conducted in saline containing bicarbonate. The worms were kept between determinations in 1 per cent saline at 37° C. as in the preceding series. The figures of Table IV show that the rate of respiration was somewhat higher than that of worms kept in pure saline. The mean respiratory quotient of all determinations in this series was 1.04 ± 0.019, identical with that found in the previous series. An aerobic acid production can therefore not be demonstrated. Stannard, McCoy and Latchford (1938) found no acid production in *Trichinella* larvae kept under aerobic or anaerobic conditions. They assume the production of as yet unidentified, but non-acidic end-products. The *Eustrongylides* larvae, however, produce, as shown previously (von Brand, 1938), considerable amounts of organic acids, if kept under strictly anaerobic conditions. Naturally the question arises, whether there is such a fundamental difference between the accessory anoxidative processes proceeding under aerobic conditions and the anaerobic processes going on, if no oxygen at all is available. The above series seems to point in this direction. I do not think, however, that this is more than a possibility, hardly as yet a probability. It should be kept in mind that the CO_2 liberated by the small amounts of acids expected would not change the RQ very much. It is furthermore entirely possible that the bicarbonate reserves of the body itself are sufficient to allow neutralization of aerobically produced acids, in which case no difference between the RQ's of worms kept in media with or without bicarbonate could be expected.

The next series was designed to ascertain whether an anaerobic period previous to the determinations would have any influence upon the gaseous exchange. Freshly isolated worms were put in glass vials of about 5 cc.

TABLE IV

Oxygen consumption and carbon dioxide production of worms kept in 1 per cent saline + 0.1 per cent sodium bicarbonate at 37° C.

Days starving	0	1	2	3	4	5	6	7
Number of determinations	12	11	10	8	8	11	8	8
O_2 cu. mm./gram/hour								
Mean	149	143	152	129	115	113	110	102
Extremes	106–206	94–193	106–185	97–198	80–157	77–154	45–240	59–184
CO_2 cu. mm./gram/hour								
Mean	158	150	158	144	121	115	111	119
Extremes	103–276	110–201	95–228	96–214	81–188	70–158	62–252	59–357
RQ								
Mean	1.05	1.06	1.03	1.11	1.03	1.00	1.01	1.06
Extremes	0.84–1.38	0.89–1.21	0.89–1.23	0.94–1.35	0.91–1.16	0.74–1.15	0.93–1.11	0.75–1.94

capacity filled with 1 per cent saline of 37° C. These were then closed with a thin slice of cork, taking care to eliminate all air bubbles between the cork and the fluid by puncturing the cork with a needle and pushing it down into the fluid. A layer of mercury was then put on top of the cork, preventing the diffusion of oxygen into the vial. It was then placed for 18 to 20 hours in an incubator at 37° C. The amount of oxygen dissolved in the saline would roughly last three-quarters of an hour to

TABLE V

Gaseous exchange of worms kept previous to the determinations for 16–18 hours under anaerobic conditions ("post-anaerobic animals" in the table) and of freshly isolated worms ("fresh animals" in the table). Only the mean values are given; the variations were of the same order as those shown in Tables I, III and IV. They were somewhat more pronounced in the "post-anaerobic" than in the "fresh" lot. The O_2 series of the "fresh animals" is the same as that given in Table I for 1 per cent saline.

Half-hour periods	1	2	3	4	5	6	7	8	9	10
Number of determinations										
Post-anaerobic animals	10	10	10	10	10	10	10	10	10	6
Fresh animals	8	8	8	8	8	8	8	8	8	8
O_2 cu. mm./gram										
Post-anaerobic animals	219	169	155	172	154	121	112	99	98	79
Fresh animals	117	92	81	78	77	80	66	70	69	74
CO_2 cu. mm./gram										
Post-anaerobic animals	164	89	96	127	122	133	127	118	107	82
Fresh animals	107	87	86	80	85	82	72	73	73	82
RQ										
Post-anaerobic animals	0.76	0.53	0.58	0.58	0.68	1.02	1.09	1.11	1.15	0.96
Fresh animals	0.90	0.91	1.04	1.03	1.10	1.04	1.08	1.05	1.04	1.10
Excess O_2 cu. mm./gram										
Post-anaerobic animals	145	95	74	104	77	41	46	29	29	5
Fresh animals	43	18	—	—	—	—	—	—	—	—
CO_2 retained cu. mm./gram										
Post-anaerobic animals	68	90	68	55	41	—	—	—	—	—
Fresh animals	17	10	—	—	—	—	—	—	—	—

one hour. Added to this is an unknown amount of oxygen present in the worm tissues and combined with the haemoglobin. After about one and one-half to two hours, the color of the worms turned to the dull red of reduced haemoglobin; the actual period of anaerobiosis was therefore about 16 to 18 hours. During this time they became invariably immotile, but they began to move again shortly after restoration of aerobic conditions. Immediately after the end of the anaerobic period, the worms were placed in the respiratory vessels (Table V,

post-anaerobic animals). The rate of oxygen consumption was very high in the beginning, if compared to that of worms taken freshly from fish (Table V, fresh animals), but then went slowly down, and after 5 hours reached about the normal level. The subtraction of the oxygen values of freshly isolated worms from those of worms previously kept anaerobically reveals a total excess oxygen consumption of 584 cu. mm. per gram. To this figure must be added 61 cu. mm. of excess oxygen found in freshly isolated worms (see below), summing up to 645 cu. mm. This is probably a minimum figure which should be raised for the 20-minute equilibration period for which no data were obtainable. Assuming the same or a slightly higher excess consumption as that found in the first half-hour period, the total excess oxygen can be assumed to lie between 750 and 800 cu. mm. per gram. This excess oxygen consumption can be interpreted in two ways. It could be a purely physical process, i.e. the amount of oxygen needed to restore an equilibrium between the liquids (haemoglobin!) and tissues of the body with the surrounding fluid. In this case the process would not have much significance. The fact, however, that the bright red color of oxyhaemoglobin reappears very quickly after restoration of aerobic conditions indicates a rapid diffusion of oxygen into the body cavity. It is probable that the 20-minute equilibration period, before the first reading was taken, was sufficient to eliminate this factor.

It seems more likely that the excess oxygen consumption represents the repayment of an oxygen debt. The oxygen missed in 16 to 18 hours of anaerobiosis amounts to 2200 to 2500 cu. mm. per gram. The observed excess oxygen consumption of 750 to 800 cu. mm. represents therefore repayment of roughly 30 per cent. Obviously, then, a large part of the oxygen debt was not repaid. The process serves to remove end-products of the anaerobic metabolism. Many free-living organisms are not able to excrete these and they accumulate in the body throughout the anaerobic period. Considerable amounts of oxygen are required during the recovery period to remove them entirely, either by partial resynthesis to glycogen or by oxidation. In frog muscle, for example, a 70 per cent repayment takes place (Rotta and Stannard, 1939) and in insects even much more oxygen is used than was missed, perhaps because a larger percentage of the acids is oxidized (Gilmour, 1940, 1941). It can be safely assumed that in the present case the relatively low percentage of oxygen repayment is correlated with the fact that the worm is able to excrete at least parts of the anaerobic end-products, as evidenced by the fact that the medium becomes markedly acid during anaerobiosis (von Brand, 1938). Obviously, all excreted acids are eliminated from

participation in any recovery process. This can involve only those end-products that are still in the body when aerobic conditions are restored.

The respiratory quotients of the worms in this series were very low during the first 2½ hours, namely 0.63 as compared to 1.06 in the following 2½ hours. In some individual cases exceedingly low values, from 0.04 to 0.08, were observed from ½ to 1½ hours after the beginning of the experiments. These low respiratory quotients represent doubtless a carbon dioxide retention. The amounts of carbon dioxide retained were calculated assuming that the actual respiratory processes also had an RQ of 1.06 during the first 2½ hours. They amounted to a total of 322 cu. mm. per gram.

It is of interest to note that the period of carbon dioxide retention was shorter than that of excess oxygen uptake (2½ against 4½ to 5 hours). The mean R.Q. after the end of the carbon dioxide retention period was, as mentioned above, 1.06,—exactly the same value that was found in the control series with freshly isolated worms (Table V, fresh animals.) This indicates that the recovery processes that are superimposed upon the normal respiratory activities must have very nearly the same RQ as the latter.

It should be noted that so far very little is known about similar processes in parasitic worms. The adult *Ascaris*, which is primarily an anaerobic living organism (von Brand, 1938*a*) shows no accumulation of an oxygen debt during anaerobiosis (Adam, 1932; Krüger, 1936). Newly-hatched *Ascaris* larvae, on the other hand, have for a short time a distinctly increased oxygen consumption that has been interpreted as the repayment of an oxygen debt incurred during development inside the egg shell (Fenwick, 1938).

The observations described above seem to open a way to decide whether the worms lead a predominantly aerobic or anaerobic life inside the fish. If the second alternative were true, one should expect that freshly isolated worms would show the same type of oxygen consumption and similar low RQ's as worms previously subjected to anaerobic conditions in vitro. There is very little evidence that such is the case, as evidenced by the experiments summarized in Table V (fresh animals). It is true that the initial oxygen uptake was slightly higher than it was later on, a fact already mentioned above in discussing the experiments summarized in Table I. The mean respiratory quotients of the first two half-hour periods with 0.90 and 0.91 respectively were somewhat lower than that of the later periods which yielded an average of 1.06. On the basis of these figures, an excess oxygen consumption of about 61 cu. mm. per gram and a carbon dioxide retention of about 27 cu. mm. can be assumed. These are very small

figures if one considers the fact that previous to the determinations the worms had doubtless lived for months in their cysts. One would have expected the repayment of a maximal debt if the oxygen supply had been restricted noticeably. This series seems therefore to establish a sound basis for the assumption that the worms are able to satisfy very nearly their entire oxygen need inside the fish. In accordance with this view is the fact that freshly isolated worms show the bright red color of oxyhaemoglobin.

Summary

1. Variations between 0 and 1.5 per cent NaCl in the medium had no influence upon the oxygen uptake of a larval *Eustrongylides,* but concentrations between 2 and 4 per cent were definitely toxic.

2. The oxygen uptake (140 cu. mm. per gram per hour) was greater than that found in *Ascaris,* but smaller than that reported for *Setaria,* hookworm or *Trichinella* larvae.

3. The oxygen uptake of nematode species of very different sizes remains more constant if calculated on the basis of weight rather than surface.

4. During a week's starvation at 37° C. the oxygen consumption decreased to about half the initial value.

5. The mean respiratory quotient of aerobically kept worms was in all series slightly above one, but no aerobic excretion of organic acids could be demonstrated with the methods employed.

6. In an aerobic period following an anaerobic period of 16 to 18 hours duration, the worms repaid about 30 per cent of the incurred oxygen debt and retained a considerable amount of carbon dioxide.

7. Freshly isolated worms, on the other hand, showed only a trace of excess oxygen consumption and carbon dioxide retainment. It is therefore concluded that the animals inside their cysts in the fish lead an almost purely oxidative life.

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